

TRIPLOIDY IN UTAH ASPEN

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The quaking aspen, *Populus tremuloides* Michx., is the most widespread tree in North America (Cottam, 1963; Fowels, 1965); a closely related species, *Populus tremula* L., occurs in Europe. The aspen in the western United States is considered by some authors (Sargent, 1961) to be a separate variety, *P. tremuloides* var. *aurea* Daniels, but most taxonomists do not accept the validity of this variety.

Aspen are normally dioecious. The flowers are minute (ca. 2 mm in diameter), unisexual, and wind pollinated. The seeds germinate soon after dispersal; but reproduction is predominantly vegetative, resulting in a clonal population structure. Shoots originate from parent roots producing characteristic "suckers." Baker (1925) and Cottam (1963) report that aspen seedlings are rare or non-existent in the Central Rocky Mountains.

Chromosome numbers reported in aspen (Darlington and Wylie, 1955) are predominantly diploid, $2n = 38$, but a few triploids, $2n = 57$, have been discovered (van Buijtenen, Joranson, and Einspahr, 1957). The same chromosome numbers were noted in the European aspen (Müntzing, 1936a), and a tetraploid, $2n = 76$, was produced experimentally (Bergström, 1940).

The present study was initiated with two principle objectives in mind. First, to determine the extent of variation, if any, of the chromosome numbers in the aspen of Utah, and second, to ascertain if a relationship existed between chromosome number and prevernal leafing patterns (Baker, 1921; Cottam, 1954). Prevernal leafing patterns are the result of differentiation in the time of leaf emergence. In some aspen clones there is a difference in emergence time of several weeks, and the variation is often striking.

MATERIALS AND METHODS

Chromosome numbers were determined primarily from meiotic material because chromosome behavior could also be analyzed, and haploid chromosome associations are easier to count than diploid complements. However, a method of obtaining mitotic chromosome numbers from leaf buds (van Buijtenen, 1957) was a useful and simple alternative to meiotic counts. Voucher specimens of each tree are deposited at the University of Utah Herbarium.

Meiosis. Pollen mother cells (PMC's) from young, developing buds of staminate trees were the source of meiotic counts. Staminate buds swell early in the fall; however, pistillate buds remain dormant until spring. During long, warm autumns, buds of male trees at low elevation may begin to open (if first subjected to an early freeze). Meiosis, how-



FIG. 1. Aspen seedling. Note the lack of rootstock attachment.

ever, appears to be delayed until the buds have undergone a few weeks of freezing temperature. Normally meiosis occurs during March and April, about a month before anthesis.

We attempted to force bud development during the winter under greenhouse conditions. Such attempts were unsuccessful in October, but were almost 100% successful from December through April. This method was also utilized by Müntzing (1936a), and van Buijtenen and Einspahr (1959) to obtain dividing nuclei.

Staminate buds undergoing meiosis varied in length from less than one centimeter to over two centimeters. However, exact bud size was not critical in this study because each inflorescence (ament) contained staminate flowers in various stages of development; the most mature anthers occurred near the base, the least mature near the apex. Because of this condition some anthers undergoing meiosis could usually be found. Whatever the bud size, meiosis appeared to be essentially complete when half the ament had emerged from the bud scales.

Early in the study buds were fixed in acetic alcohol (1:3) for 12–24 hours. However, chromosomes were obscured by what appeared to be oil droplets. This problem was alleviated by changing the fixative to

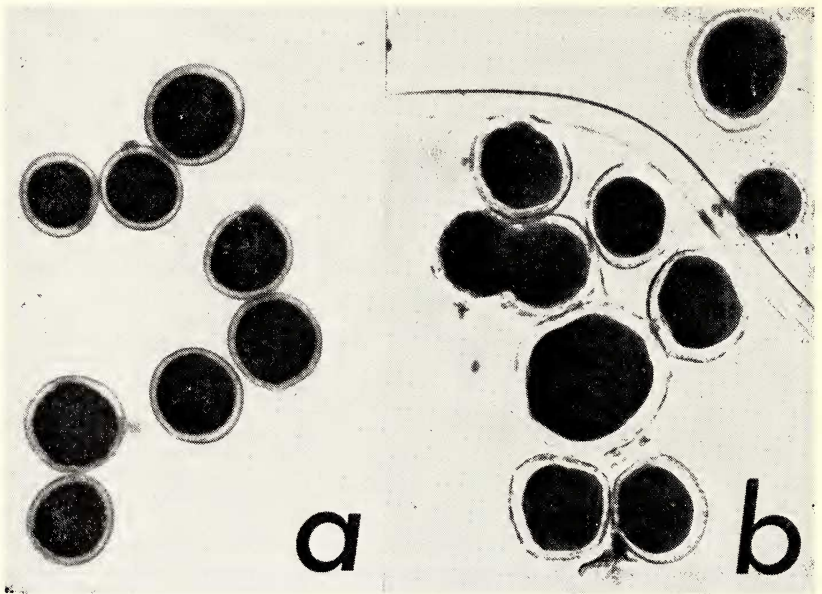


FIG. 2. Aspen pollen, $\times 400$: 2a, single size class; 2b, two size classes.

chloroform, absolute ethyl alcohol, and glacial acetic acid (3:2:1), and then rinsing several times in distilled water and in 70% ethyl alcohol. Buds stored in 70% ethyl alcohol at -10°C remained in workable condition for several months.

A drop of ferric acetate was added to each vial of fixative as a mordant. This procedure noticeably improved chromosomal staining. Anthers were squashed and stained in heated acetocarmine and observed under phase contrast microscope. Chromosome numbers were recorded with the aid of a camera lucida and photomicrographs.

Mitosis. Mitotic chromosome numbers were obtained from young leaves just emerging from the bud. The technique was described by van Buijtenen (1957), but modified in this study. The basis of his technique was a three hour pre-treatment in saturated aqueous paradichlorobenzene (PDB) at 37°F , which we also employed. However, after the pre-treatment in PDB, the leaf buds were treated as previously described for the anthers, except the tissue was softened in 20% HCl for one minute before squashing.

An alternate source of mitotic material was obtained by planting pieces of aspen rootstock three to four inches long. Young shoots usually emerged in a few days, and these were treated in the same manner as leaf buds. This technique extended the period during which mitotic material could be obtained.

Pollen. Whole aments were collected from diploid trees in anthesis,

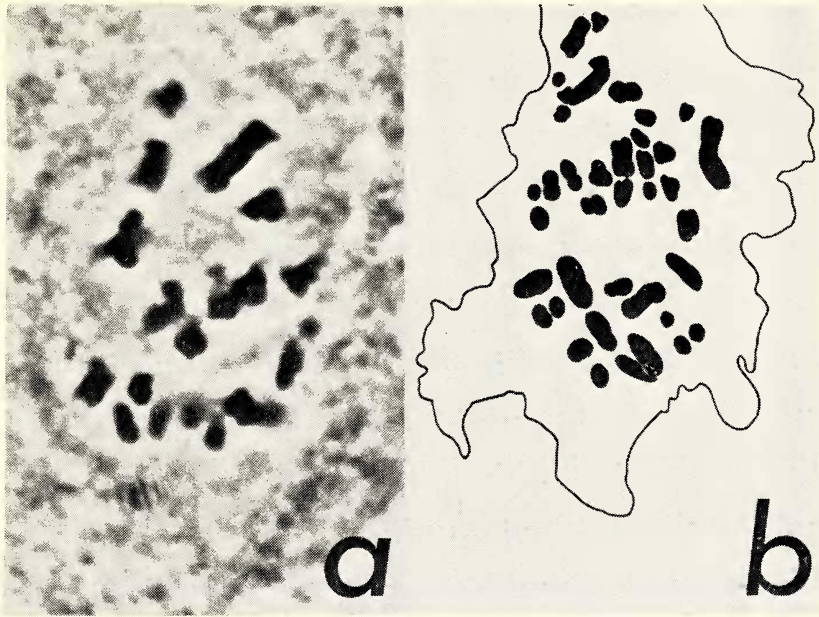


FIG. 3. Diploid aspen: 3a, meiotic metaphase I, $n = 19$, $\times 2400$; 3b, mitotic metaphase, $2n = 38$ $\times 4000$.

dried, and stored in envelopes. The diploid pollen was studied to determine if large (unreduced?) pollen grains were produced (Müntzing, 1936a), which might indicate a possible mechanism for the origin of triploidy. Stainability (in Cotton Blue and lactophenol) was used as an indicator of pollen viability (Davis and Heywood, 1963). Observations and photomicrographs were made under bright field microscopy.

RESULTS

Seedlings. While collecting sections of rootstock for mitotic chromosome material, five putative seedlings were found (fig. 1). These came from four widely separated areas and represent about 10% of the saplings studied, suggesting that the occurrence of seedlings is more frequent than previously believed. Even so, most aspen reproduction in this area is still by "suckering" from rootstocks.

Pollen. Pollen from a number of widely separated diploid trees was examined for stainability and size. The variation in both characteristics was similar to that reported in European aspen by Johnsson (1940). Pollen from some trees was relatively uniform. Others contained pollen with a wide range of sizes, and a few appeared to have two size classes. One size class (ca. 30μ) varied around the normal diploid dimensions as established by Johnsson (1940). The other size class (unreduced?) varied around 50μ . Stainability also varied, the percentage of deeply

TABLE 1. CHROMOSOME NUMBERS IN UTAH POPULATIONS OF *POPULUS TREMULOIDES*. Numbers in parentheses after elevation indicate the number of trees counted.

Cache Co.: Logan Canyon, 6500 ft. (1), $n = 19$. Carbon Co.: Price Canyon, 6800 ft (2), $n = 19$. Salt Lake Co.: Alta, 9600 ft (1), $n = 28$; Alta, 9500 ft (2), $2n = 38$; Brighton, 8200 ft (3), $n = 19$; Brighton, 8400 ft (1), $n = \text{ca. } 38$; Brighton, 8400 ft (2), $2n = 38$; Lambs Canyon, 7000 ft (1), $n = 19$; Lambs Canyon, 7500 ft (1), $2n = 57$; Parleys Canyon, 5700 ft (1), $n = 29$; Parleys Canyon, 5700 ft (4), $n = 19$; Parleys Canyon, 5700 ft (2), $2n = 38$. San Juan Co.: 2 mi N Monticello, 7000 ft (1), $n = 19$. Tooele Co.: South Willow Creek Canyon, 6000 ft (1), $n = 19$. Utah Co.: American Fork Canyon, 7000 ft (2), $n = 19$; American Fork Canyon, 9500 ft (1), $2n = 38$; Spanish Fork Canyon 7200 (1), $2n = 38$. Wasatch Co.: Soapstone Junction, 8500 ft (1), $n = 19$.

stained grains ranging from about 30% to about 95%. Because of the small number of pollen grains in the 50μ size class, no meaningful comparisons of stainability between size classes was possible. Many of the pollen grains in the large size class were densely stained, suggesting that they were viable. Figure 2 illustrates these size classes.

Chromosomes. The results of the chromosome studies are presented in Table 1. Chromosome numbers from 28 trees of 18 populations were obtained. Both mitotic ($2n$) chromosome numbers and meiotic (n) numbers were determined from several populations, but no specific number of counts was necessarily made for any population, although generally the polyploid numbers were better documented than diploid counts. Specific locations for each population are given in Table 1.

Due to the small size and large number of chromosomes, aspen chromosomes are difficult to photograph because some chromosomes are usually out of the plane of focus. Therefore, some of the photographs are accompanied by drawings, and in some cases only drawings are presented (figs. 3, 4, 5). Also for these reasons, the supposed tetraploid is reported only as an approximate count (table 1). Meiosis appeared to be irregular in the putative tetraploid and good chromosome spreads were not obtained. Further efforts to confirm this chromosome number are needed.

DISCUSSION

Triploidy has been reported in quaking aspen, but the incidence is low. Van Buijtenen, et al. (1957) cited only four triploid populations, one from Minnesota, two from Michigan, and one from Colorado. Among the 18 populations we studied (table 1) all were diploid except for three triploids, $n = 28, 29$; $2n = 57$, and an apparent tetraploid, $n = \text{ca. } 38$ (fig. 5).

Therefore, the Wasatch Mountains of Utah may contain a higher proportion of polyploids than areas previously studied. However, because of the relatively small sample the apparent high proportion of triploids could be misleading.

Origin of Polyploidy. The first occurrence of polyploidy (triploidy) in aspen was reported by Müntzing (1936a), who attributed the origin of

triploidy in European aspen to the union of an unreduced ($2n$) gamete with a normal (n) gamete. Müntzing noticed irregular meiosis in PMC's of triploids, and postulated that in reduced ($3n$) pollen from this aberrant meiosis could fertilize a normal (n) egg resulting in a tetraploid. Such a tetraploid was produced experimentally (Bergström, 1940).

Pollen from both diploid and triploid European aspen exhibited wide variation in size (Johnsson, 1940). In triploids, two size classes of pollen were reported by Müntzing (1936a), but the pollen from triploids studied by Johnsson (1940) showed continuous variation. However, occasional bimodal distribution of pollen size was not discounted by Johnsson.

The pollen analyzed in the present study (fig. 2) followed the patterns reported for European aspen. Pollen from diploid trees showed wide variation in size, and a few cases exhibited bimodal size distribution. If these criteria are valid the evidence supports the occurrence of unreduced ($2n$) pollen in Utah aspen. However, pollen from known triploid trees has not been studied as a basis for comparison, nor have pollen grain mitoses been examined to demonstrate the actual occurrence of $2n$ pollen.

Regardless of these considerations, Müntzing's proposal that polyploids in aspen are of autopoloid origin is the most acceptable. However, these are probably inter-populational autopoloids. Two additional origins of autopoloidy, however, are theoretically possible, viz., somatic mutation and unreduced female gametes. Somatic mutation could feasibly occur during suckering, but triploidy would be difficult to envision by this process. Unreduced eggs are considered by Cheeseman and Dodds (1942) to be responsible for the origin of triploidy in bananas. The same phenomenon may occur in aspen, and that possibility should be examined.

The characteristics of autopoloids outlined by Müntzing (1936b) and Stebbins (1950) seem to be applicable to quaking aspen. In meiosis, multivalent formation occurs. One triploid (fig. 4) contains three distinct trivalents at metaphase I. These are similar in shape to those reported by Müntzing (1936a) from European aspen. The presumed tetraploid contains at least one typical quadrivalent (fig. 5).

The morphological differences between polyploid and diploid trees are slight and subtle. Van Buijtenen, et al. (1957) report that triploid aspen are generally larger than comparable diploids, especially the leaves. No such distinction could be attributed to the polyploids in this study due to the extreme leaf variation in adjoining diploid clones (often more than a twofold size difference).

In the European aspen, tetraploid trees were experimentally produced from diploid-triploid crosses (Bergström, 1940). The chances are far greater for the same thing to occur in nature because much larger samples occur. Yet tetraploidy has not been reported. Johnsson (1942) explains the lack of natural tetraploids in *Populus* by suggesting that triploidy may be the optimum level of polyploidy in this genus. But

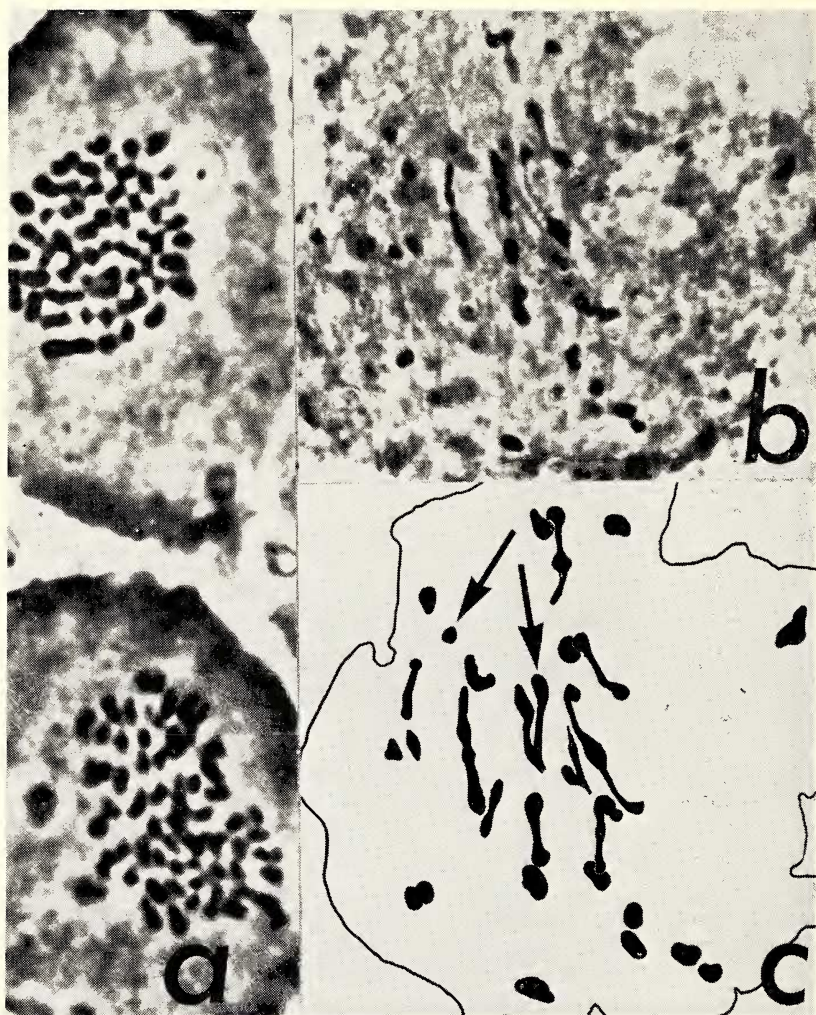


FIG. 4. Triploid aspen: 4a, mitotic metaphase, $2n = 57 \times 2880$; 4b, meiotic metaphase I, $n = 28 \times 1900$; 4c, camera lucida interpretation of 4b, 3 III, 22 II, 3 I, arrows indicate typical trivalent and univalent.

other groups of plants often show much higher levels of optimum polyploidy (Avery, et al., 1959). Often the optimum ploidy level depends on the size of original base chromosome number.

Since tetraploids apparently occur in nature, another natural route to triploidy is possible. The cross between a tetraploid and a diploid would yield a triploid, but this is probably secondary to the origin by unreduced $2n$ pollen. However, if tetraploids exist in an area, then triploids should be more common. This could partially explain the high incidence of triploidy reported in this study.

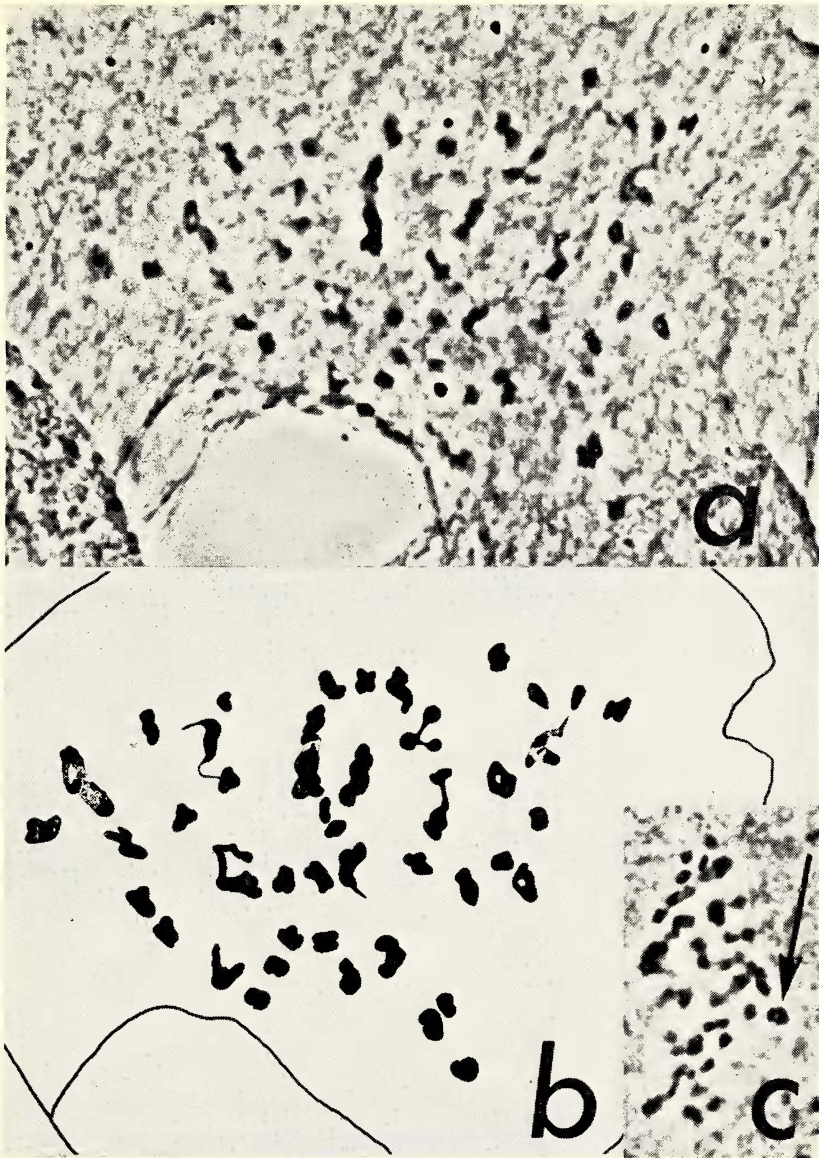


FIG. 5. Putative tetraploid aspen: 5a, meiotic metaphase I, $n = \text{ca. } 38 \times 2240$; 5b, camera lucida interpretation of 5a; 5c, quadrivalent in putative tetraploid denoted by arrow, $\times 2240$.

Seedlings and Ecology. Sexual reproduction is necessary for production of autopolyploids through unreduced gametes. However, Baker (1918; 1925) and Cottam (1963), after extensive searches, report that they found no aspen seedlings in Utah. Ellison (1943) and Larson (1944)

each report that seedlings occur—but rarely. Kimball Harper (pers. com.) discovered a group of aspen seedlings still in the cotyledon stage. In our study five seedlings were found. We distinguished these as seedlings because of the absence of root attachments to any other aspen.

Baker and Cottam each suggest that the biggest single factor preventing seedling establishment is the early summer drought common to Utah. Perhaps polyploid seedlings are better able to withstand desiccation. A study of aspen seedlings combining cytology and ecology might yield an answer to this question. Periodic seedling establishment probably occurs in unusually wet years.

No ecological correlations, other than the above, can be proposed because of the seemingly random distribution of the polyploids. One triploid was found near the lower elevational limits of aspen, one slightly higher, and one very high. Also one triploid and the tetraploid are correlated with the prevernal leafing patterns observed by Cottam (1954). But one prevernal leafing aspen was clearly diploid, and one triploid aspen was definitely late-leafing.

The Parleys Canyon population of aspen has been especially interesting. A single triploid occurred here. However, later collections from nearby trees were diploid with both staminate and pistillate trees present. Several of the trees taken for pistillate at first were obviously hermaphroditic on closer inspection. Unlike the hermaphrodites reported by Pauley and Mennel (1957) and Santamour (1956), these trees contained almost entirely bisexual flowers. One of these was a diploid $2n = 38$. These were the only cases of bisexual flowers found in our collections. Among predominantly staminate trees no bisexual flowers were observed, though Pauley and Santamour each reported a low percentage of hermaphrodites in their studies. This same small area also contained two of the seedlings reported. The aspen occur here about 100 feet above the creek on an east facing slope. Additional moisture from seeps or springs along this hillside may have provided favorable conditions for seedling establishment.

This paper is abstracted from a thesis submitted by the first author in partial fulfillment of the M.S. degree at the University of Utah. His present address is: Department of Botany, University of Washington, Seattle, Washington.

LITERATURE CITED

- AVERY, A. G., and S. SATINA, and J. RIETSEMA. 1959. *Blakeslee: The genus Datura*. Ronald Press, New York.
- BAKER, F. S. 1918. Aspen reproduction in relation to management. *J. Forest.* (Washington) 16:389-398.
- . 1921. Two races of aspen. *J. Forest.* (Washington) 19:412-413.
- . 1925. Aspen in the Central Rocky Mountain Region. *U. S. D. A. Bull.* 1291.
- BERGSTROM, I. 1940. On the progeny of diploid \times triploid *Populus tremula*. With special reference to the occurrence of tetraploidy. *Hereditas* 26:191-201.
- CHEESEMAN, E. E., and K. S. DODDS. 1942. Genetical and cytological studies of

- Musa IV. Certain triploid clones. *J. Genet.* 43:337-357.
- COTTAM, W. P. 1954. Prevernal leafing aspen in Utah Mountains. *J. Arnold Arbor.* 35:239-248.
- . 1963. Quaking aspen (*Populus tremuloides* Michx.). *Naturalist* 14:3-15.
- DARLINGTON, C. D., and A. P. WYLIE. 1955. Chromosome atlas of flowering plants. Allen and Unwin, London.
- DAVIS, P. H., and V. H. HEYWOOD. 1963. Principles of angiosperm taxonomy. Van Nostrand, Princeton.
- ELLISON, L. 1943. A natural seedling of western aspen. *J. Forest.* (Washington) 41:767-768.
- FOWELS, H. A. 1965. Silvics of forest trees of the United States. U. S. D. A. Handb. 271.
- JOHNSON, H. 1940. Cytological studies of diploid and triploid *Populus tremula* and of crosses between them. *Hereditas* 26:321-352.
- . 1942. Cytological studies of triploid progenies of *Populus tremula*. *Hereditas* 27:306-312.
- LARSON, G. C. 1944. More on seedlings of western aspen. *J. Forest.* (Washington) 42:452.
- MÜNTZING, A. 1936a. The chromosomes of a giant *Populus tremula*. *Hereditas* 21:383-393.
- . 1936b. The evolutionary significance of autopolyploidy. *Hereditas* 21:263-378.
- PAULEY, S. S., and G. F. MENNEL. 1957. Sex ratio and hermaphroditism in a natural population of quaking aspen. *Minnesota Fores. Notes* 55.
- SANTAMOUR, F. S. 1956. Hermaphroditism in *Populus*. *Proc. Third Northeast Forest Tree Impr. Conf.*
- SARGENT, C. S. 1961. *Manual of the trees of North America*. Dover Publications, New York.
- STEBBINS, G. L. 1950. *Variation and evolution in plants*. Columbia Univ. Press, New York.
- VAN BUIJTENEN, J. P. 1957. A technique for fixing and staining mitotic chromosomes in aspen. *Forest Sci.* 5:48.
- , P. N. JORANSON, and D. W. EINSPAHR. 1957. Naturally occurring triploid quaking aspen in the United States. *Proc. Soc. Amer. Foresters Ann. Meeting*, Syracuse, New York.
- , and D. W. EINSPAHR. 1959. Note on the presence of sex chromosomes in *Populus tremuloides*. *Bot. Gaz.* 121:60-61.

NOMENCLATURE AND INTERPRETATION OF A CALIFORNIA SUBSPECIES IN ARCTOSTAPHYLOS (ERICACEAE)

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McMinn (1939), having had access to Adams' specimens and doctoral dissertation (1935), published *Arctostaphylos tomentosa* (Pursh) Lindl. var. *crinita* Adams ex McMinn with a Latin diagnosis ("A specie differt: ramis junioribus tomentosis et albo-divaricate-crinitis; corticis levigatis.") Adams (1940) published the same variety as *A. crustacea* Eastw. var. *tomentosiformis* Adams for which he cited as type *Adams 928* (UC